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ORIGINAL RESEARCH

A Genome-Wide Association Study of Bisphosphonate-Associated Atypical Femoral Fracture

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Abstract

Atypical femoral fracture is a well-documented adverse reaction to bisphosphonates. It is strongly related to duration of bisphosphonate use, and the risk declines rapidly after drug withdrawal. The mechanism behind bisphosphonate-associated atypical femoral fracture is unclear, but a genetic predisposition has been suggested. With the aim to identify common genetic variants that could be used for preemptive genetic testing, we performed a genome-wide association study. Cases were recruited mainly through reports of adverse drug reactions sent to the Swedish Medical Products Agency on a nation-wide basis. We compared atypical femoral fracture cases ($n = 51$) with population-based controls ($n = 4891$), and to reduce the possibility of confounding by indication, we also compared with bisphosphonate-treated controls without a current diagnosis of cancer ($n = 324$). The total number of single-nucleotide polymorphisms after imputation was 7,585,874. A genome-wide significance threshold of $p < 5 \times 10^{-8}$ was used to correct for multiple testing. In addition, we performed candidate gene analyses for a panel of 29 genes previously implicated in atypical femoral fractures (significance threshold of $p < 5.7 \times 10^{-6}$). Compared with population controls, bisphosphonate-associated atypical femoral fracture was associated with four isolated, uncommon single-nucleotide polymorphisms. When cases were compared with bisphosphonate-treated controls, no statistically significant genome-wide association remained. We conclude that the detected associations were either false positives or related to the underlying disease, i.e., treatment indication. Furthermore, there was no significant association with single-nucleotide polymorphisms in the 29 candidate genes. In conclusion, this study found no evidence of a common genetic predisposition for bisphosphonate-associated atypical femoral fracture. Further studies of larger sample size to identify possible weakly associated genetic traits, as well as whole exome or whole-genome sequencing studies to identify possible rare genetic variation conferring a risk are warranted.

Keywords Genome-wide association study · Atypical fractures · Bisphosphonate · Drug-related side effects and adverse reactions · Pharmacogenetics

Introduction

For over a decade, atypical fracture of the femoral bone (AFF) has been a well-documented adverse drug reaction (ADR) associated with long-term bisphosphonate use [1]. AFF is normally preceded by weeks or months of thigh pain and is in contrast to ordinary fragility fractures related to no or minimal trauma [2]. The term 'atypical' refers to the deviant transverse pattern of the fracture-line revealed on plain radiographs of the affected femur [2]. Although not all AFFs occur after bisphosphonate exposure, there is a strong correlation with duration of bisphosphonate use. A more than 100-fold increase in risk is seen after 4–5 years

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of bisphosphonate use, and the risk declines rapidly after cessation of treatment [3–5].

By now, clinicians, the scientific community and patients have come to realize the many challenges associated with AFFs. Over the last decade, a 50% decrease in prescriptions of bisphosphonates for primary and secondary prevention of fragility fractures has been seen [6]. This significant decline in preventive medication is believed to be due to fear of ADRs.

A major challenge in the prevention of AFF is the overall lack of knowledge about the mechanism behind this fracture type. Theories highlight long-term buildup of micro-cracks in the bone due to an over-suppression of bone remodeling that eventually leads up to failing skeletal integrity and stress fractures [7]. Predisposing risk factors are long-term use of bisphosphonates [3], female sex [3, 8], Asian ethnicity [9], and bowing of the femur [10]. Since only a minority of bisphosphonate users develop AFF, pathophysiological theories include a predisposing genetic trait, altered collagen cross-linking, accumulation of microdamage, increased mineralization, reduced heterogeneity of mineralization, variation in rates of bone turnover, and reduced vascularity [2].

A recent systematic review found six published studies that investigated the role of genetics on AFF in a total of 44 patients [11]. The review also identified 23 cases of AFF associated with seven different monogenetic bone disorders, of which seven cases had been exposed to a bisphosphonate. There is thus some evidence of rare genetic susceptibility loci for bisphosphonate-associated AFF. If common risk variants, i.e., genetic variants occurring among at least 1%, also exist, as has been shown for many rare adverse drug reactions [12], it might be feasible to predict patients at risk through preemptive genotyping. We performed the largest case–control GWAS to date, to determine whether common genetic variants contribute to risk of bisphosphonate-associated AFF. We also performed candidate gene analyses of 29 genes that have been implicated in AFF [11].

Materials and Methods

Sample Description

The basis for case recruitment was through nation-wide spontaneous ADR reports sent from healthcare professionals to the Swedish Medical Products Agency between the years 2006 and 2015. Each patient should be at least 18 years of age and able to give informed consent. Case definition for AFF was according to the American Society for Bone and Mineral Research [2].

We collected clinical data (demographics, medical history, drug treatment history, X-ray images, and ancestry) through interviews using a standardized questionnaire, and by obtaining and reviewing medical records. Prior to genetic analysis, each case including radiographs was evaluated by at least one senior consultant in orthopedics.

Overall, 71 reported cases were available. Of these, 18 cases were not possible to include (five were deceased, five could not be reached, four declined to participate, two were not suitable to be contacted according to the reporting physician, one was not able to perform the interview, and in one case the reporting physician could not be reached). Of the remaining 53 cases, two did not pass radiograph adjudication (ordinary fragility fractures) and therefore 51 cases, all with complete fractures, were included in the study. We compared the cases with two sets of controls. In the main analysis, we utilized 4891 population controls from the Swedish Twin Registry [13], all non-related individuals. The proportion of women in this population was 46%, and birth years ranged from 1911 to 1958 (1911–1919, 0.78%; 1920–1929, 10.3%; 1930–1939, 27.7%; 1940–1949, 45.7%; 1950–1958, 15.5%). Information on diseases and drug treatments for controls was available by linkage to individual data from the Swedish National Patient Register and the Swedish Prescribed Drug Register. Complete linkage is enabled by use of the individual personal registration number provided to all Swedish citizens. To determine whether any positive GWAS findings might be due to confounding by indication, we also defined a matched control group, consisting of patients who had collected at least one prescription of a bisphosphonate and who did not have a current cancer diagnosis. This gave a total of 324 controls that had been prescribed bisphosphonates and thus resembling the same source population of individuals as the cases, i.e., bisphosphonate users. Four out of five matched controls were women, which corresponds well with the overall proportion of women/men prescribed bisphosphonates in Sweden according to the Swedish Prescribed Drug Register. None of the cases with AFF had a current diagnosis of cancer.

Genome-Wide Array Data and Analyses

DNA was extracted from peripheral venous blood. Cases were genotyped with the Illumina Infinium OmniExpressExome 1 M array, and controls were genotyped with the Illumina HumanOmniExpress 700 K array. Genotype calls were generated using the Genome Studio software from Illumina and the Genome Reference Consortium human assembly GRCh37.

Genotyping quality control (QC) and data management was performed using PLINK v1.9 [14]. The resulting merged

data included 604,238 SNPs post QC. Imputation was performed using the Sanger imputation server [15]. The pipeline with Eagle2 (v2.0.5) prephasing [16] and PBWT imputation [17] was used with the haplotype reference consortium panel as reference (v1.1) [15]. The total number of SNPs after imputation and QC was 7,585,874. All cases and controls were within the European cluster according to genetic principal component analysis (PCA), except for one case of Chilenean origin (Supplemental Fig. 1). Additional details on QC, PCA and imputation can be found in the Supplement.

Logistic regression on a genome-wide level was performed using PLINK v1.9 [14]. All genome-wide analyses were adjusted for the first four principal components. SNP effects were modeled only as additive and the conventional genome-wide significance threshold $p < 5 \times 10^{-8}$ was used to correct for multiple testing [18]. Results are presented as Manhattan plots. QQ-plots are presented in Supplemental Figs. 2 and 3.

Candidate Gene Analyses

In addition to genome-wide analyses, we performed candidate gene analyses in the imputed data set for a panel of 29 genes that have been implicated in AFF (Table 1) [11]. We examined a panel consisting of 8709 SNPs distributed in these genes. We both tested all 51 cases vs all 4891 controls and all 51 cases vs the 324 matched controls. Adjustment for multiple testing was done with Bonferroni correction ($0.05/8709 \approx 5.74 \times 10^{-6}$).

Power Calculation

Given a genome-wide significance level of $p < 5 \times 10^{-8}$ and using an additive genetic model, our sample size was powered to detect common genetic variants with effect sizes of clinical utility [19]. We had approximately 80% power to detect an odds ratio (OR) of 3–4 for variants with a minor allele frequency (MAF) of 40%, and 80% power to detect an OR of 4–5 for variants with a MAF of 20% (Supplemental Figs. 4 and 5). Given the significance level of $p < 5.74 \times 10^{-6}$ in the candidate gene analyses, we had 80% power to detect an OR of about 3 for variants with a MAF of 40%, and 80% power to detect an OR of about 4 for variants with a MAF of 20% (Supplemental Figs. 6 and 7).

Results

Characteristics of the 51 cases (48 women and 3 men) of bisphosphonate-associated AFF and the 324 matched controls are shown in Table 2. Most of the cases were of Swedish ethnicity ($n = 47$), while one each was of Finnish, Norwegian, British or Chilean origin.

Table 1 Candidate genes tested in the study

Gene	Chromosome	Start position	End position
ACKR3 (CXCR7)	2	237476430	237491001
ACOXL	2	111490150	111875799
ALPL	1	21835858	21904905
CCDC147	10	106113522	106214848
CNGB1	16	57917503	58005020
COL1A2	7	94023873	94060544
CRYBB2	22	25615489	25627836
CTSK	1	150768684	150780799
CYP1A1	15	75011883	75017951
DOCK2	5	169064251	169510386
EDC3	15	74922899	74988633
FN1	2	216225163	216300895
FOXK2	17	80477589	80602538
GGA3	17	73232694	73258444
GGPS1	1	235490665	235507847
HHAT	1	210501596	210849638
LIPN	10	90521163	90537999
MVD	16	88718343	88729569
NAT8B	2	73927636	73928467
NGEF	2	233743396	233877982
OR2L13	1	248100493	248264224
OR51T1	11	4903049	4904113
PCK2	14	24563262	24579807
PPEF2	4	76781020	76823724
SF3B3	16	70557691	70608820
SLC15A5	12	16341419	16430619
SLC2A6	9	136336217	136344259
SYDE2	1	85622556	85666729
SYTL2	11	85405267	85522184

Genes implicated in atypical femoral fractures [11]

Genome-Wide Association Analyses—Cases Versus All Population Controls

Bisphosphonate-associated AFF was significantly associated with four isolated single nucleotide polymorphisms (SNP) (Fig. 1a; Table 3). The first SNP was rs7729897, which is located in an intergenic region upstream of the NR3C1 gene (nuclear receptor subfamily 3 group C member 1) on chromosome 5, OR 10.27 [95% confidence interval (CI) 4.95, 21.31] $p = 4.00 \times 10^{-10}$. The NR3C1 gene encodes a glucocorticoid receptor, which functions as a transcription factor that activates glucocorticoid responsive genes, and as a regulator of other transcription factors [20]. Variants of this gene have been associated with decreased bone mineral density in patients with endogenous hypercortisolism [21, 22].

The second SNP was rs11465606 positioned in an intron within the IL18R1 gene (interleukin 18 receptor 1) on chromosome 2, OR 6.15 [95% CI 3.32, 11.37], $p = 7.13 \times 10^{-9}$. The third SNP was rs145787127, which is located in an intron of the NTN1 (netrin 1) gene on chromosome 17, OR 7.37 [95% CI 3.63, 14.93], $p = 3.08 \times 10^{-8}$. Genetic variation within NTN1 has been linked to osteoporosis [23]. The last SNP was rs144094653, located close to the pseudogene TUBB8P5 (tubulin beta 8 class VIII pseudogene 5 on chromosome 12, OR 7.68 [95% CI 3.70, 15.91], $p = 4.20 \times 10^{-8}$.

Genome-Wide Association Analyses—Cases Versus Controls with Bisphosphonate Use

No statistically significant association with gene status was revealed when cases of bisphosphonate-associated AFF were compared with matched controls (Fig. 1b; Table 4).

Candidate Gene Analyses—Cases Versus All Population Controls

When cases of bisphosphonate-associated AFF were compared with all population controls, there were no statistically significant associations (Fig. 2a; Table 5; Supplemental Table 1).

Fig. 1 **a** Manhattan plot of the genome-wide association analysis—cases vs all controls. **b** Manhattan plot of the genome-wide association analysis—cases vs matched controls. Analyses of 51 cases of bisphosphonate-associated atypical femoral fractures versus **a** all 4891 population controls, and **b** 324 matched controls. There were 7,585,874 SNPs after imputation, and adjustment was made for genetic principal components 1–4. The red line shows the threshold for genome-wide significance of 5×10^{-8}. **a** Four SNPs were statistically significant when cases were compared with all 4891 controls. The top SNP was rs7729897, located in an intergenic region upstream of the NR3C1 gene (nuclear receptor subfamily 3 group C member 1) on chromosome 5, odds ratio (OR) 10.27 [95% confidence interval (CI) 4.95, 21.31] $p = 4.00 \times 10^{-10}$. There was also a significant association with rs11465606 positioned in an intronic region within the IL18R1 gene (interleukin 18 receptor 1) on chromosome 2, OR 6.15 [95% CI 3.32, 11.37], $p = 7.13 \times 10^{-9}$. A third significant association was with rs145787127, which is located in an intron region of the NTN1 (netrin 1) gene on chromosome 17, OR 7.37 [95% CI 3.63, 14.93], $p = 3.08 \times 10^{-8}$. The fourth significant association was with rs144094653, located close to the pseudogene TUBB8P5 (tubulin beta 8 class VIII pseudogene 5 on chromosome 12, OR 7.68 [95% CI 3.70, 15.91], $p = 4.20 \times 10^{-8}$. *SNP* single nucleotide polymorphism. **b** There were no statistically significant findings when cases were compared with matched controls. *SNP* single nucleotide polymorphism

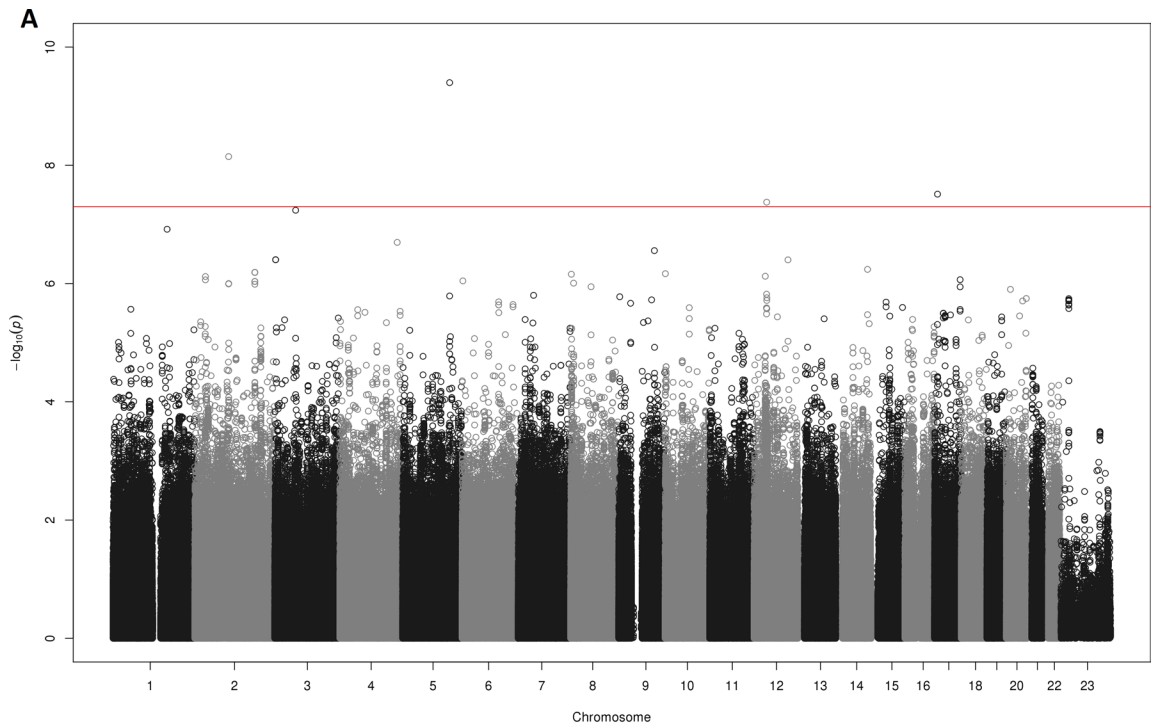
Table 2 Characteristics of cases of bisphosphonate-associated atypical femoral fractures and matched controls

	AFF ($n = 51$)	Matched controls ($n = 324$)
Gender (*n* female, [proportion female])	48 [0.94]	257 [0.79]
Age[a] (mean, years [range])	70.7 [47-86]	71.5 [52-93]
PPI (*n*, [proportion])	17 [0.33]	100 [0.31]
Systemic corticosteroids (*n*, [proportion])	17 [0.33]	123 [0.38]
Alendronic acid (*n*, [proportion])	47 [0.92]	264 [0.81]
Zoledronic acid (*n*, [proportion])	2 [0.039]	4 [0.012]
Risedronic acid (*n*, [proportion])	4 [0.078]	51 [0.16]
Etidronic acid (*n*, [proportion])	0 [0]	7 [0.022]
Ibandronic acid (proportion)	0 [0]	1 [0.0031]
Clodronate (proportion)	0 [0]	0 [0]
Oral administration (proportion)	49 [0.96]	320 [0.99]
Indication for treatment with bisphosphonate		Unknown
Osteoporosis (*n*)	45	
Prophylaxis due to corticosteroid treatment (*n*)	2	
Unknown (*n*)	4	
Fracture location		N/A
Femur (*n*)	51	

Matched controls were individuals who had collected at least one prescription of a bisphosphonate. We excluded as matched controls those individuals who had a diagnosis of cancer (any type) 12 months prior to or following first collection of a prescription of a bisphosphonate. Note that some patients have received more than one bisphosphonate

[a]Age at time of onset of AFF for cases, and time of first recorded collection of a prescription of a bisphosphonate for controls

AFF atypical femoral fractures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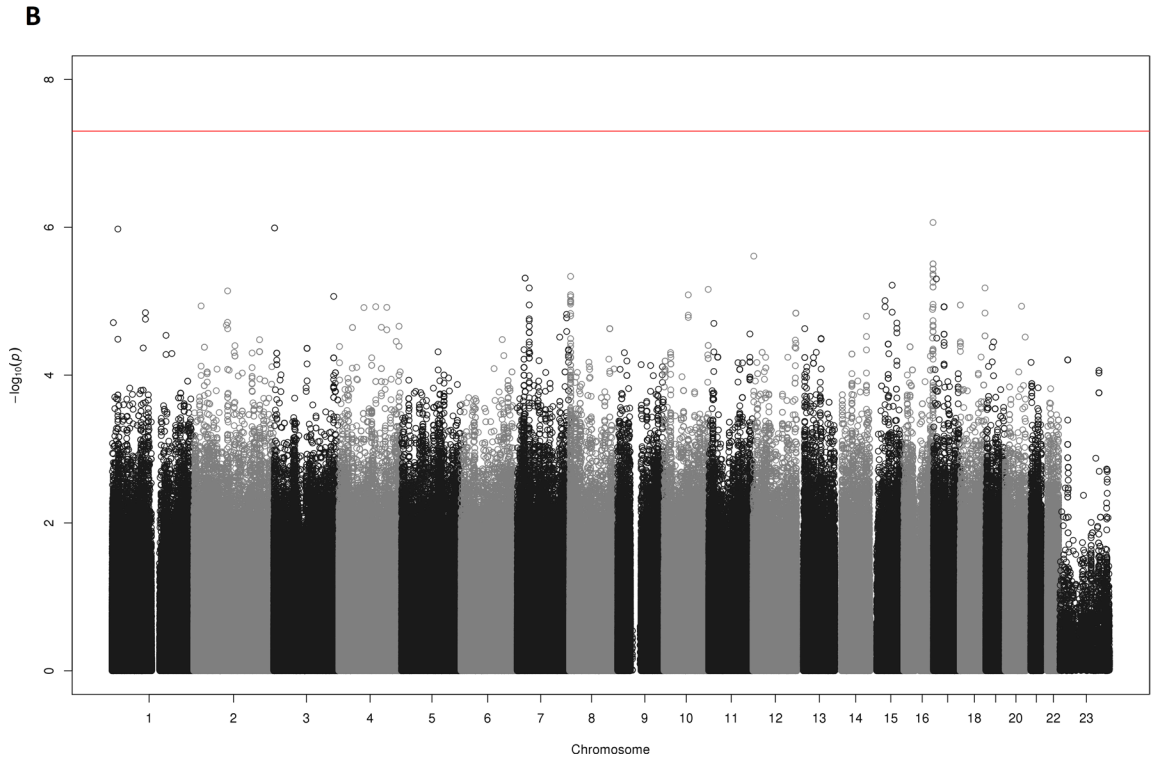
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Table 3 Top genome-wide associations with bisphosphonate-associated atypical femoral fractures

CHR	SNP	BP	Minor allele	N	OR	L95	U95	p	GTPS	MAF cases	MAF controls	Gene
5	rs7729897	142970862	G	4942	10.27	4.949	21.31	4.000×10^{-10}	G/C	0.098	0.01	
2	rs11465606	102988300	A	4942	6.149	3.324	11.37	7.131×10^{-9}	A/C	0.128	0.024	IL18R1
17	rs145787127	9142414	A	4942	7.366	3.633	14.93	3.076×10^{-8}	A/G	0.098	0.016	NTN1
12	rs144094653	38593619	A	4942	7.675	3.704	15.91	4.201×10^{-8}	A/G	0.088	0.014	
3	rs73111385	63645410	G	4942	5.042	2.811	9.045	5.755×10^{-8}	G/A	0.137	0.031	SNTN
1	rs113093597	165017843	A	4942	6.144	3.137	12.03	1.205×10^{-7}	A/G	0.098	0.017	
4	rs191328328	174611710	C	4942	8.951	3.917	20.46	2.013×10^{-7}	C/T	0.069	0.009	
9	rs12336042	108538200	A	4942	6.731	3.252	13.93	2.774×10^{-7}	A/T	0.088	0.015	TMEM38B
3	rs76646538	2694727	C	4942	3.933	2.317	6.676	3.950×10^{-7}	C/T	0.157	0.04	CNTN4
3	rs6768500	2693258	C	4942	3.932	2.316	6.675	3.962×10^{-7}	C/G	0.157	0.04	CNTN4
12	rs147502517	103265420	T	4942	7.191	3.354	15.42	3.972×10^{-7}	T/G	0.078	0.012	PAH
14	rs72698961	96278663	G	4942	5.128	2.701	9.734	5.762×10^{-7}	G/A	0.118	0.027	
2	rs74476239	182754649	C	4942	6.925	3.232	14.84	6.477×10^{-7}	C/T	0.078	0.012	
2	rs78658531	182741934	G	4942	6.925	3.232	14.84	6.477×10^{-7}	G/A	0.078	0.012	
2	rs78797265	182736267	T	4942	6.925	3.232	14.84	6.477×10^{-7}	T/G	0.078	0.012	
2	rs78890965	182734044	T	4942	6.925	3.232	14.84	6.477×10^{-7}	T/C	0.078	0.012	
10	rs112889159	899303	T	4942	8.161	3.564	18.69	6.807×10^{-7}	T/A	0.069	0.01	LARP4B
8	8:2410672	2410672	T	4942	7.257	3.318	15.88	6.952×10^{-7}	T/C	0.078	0.013	
12	rs116973965	34352942	A	4942	8.121	3.542	18.62	7.524×10^{-7}	A/G	0.069	0.011	
2	rs56272862	32379663	G	4942	3.995	2.307	6.917	7.610×10^{-7}	G/A	0.157	0.045	SPAST
17	17:77861401	77861401	T	4942	7.375	3.328	16.34	8.604×10^{-7}	T/G	0.069	0.01	
2	rs72796871	32393157	A	4942	3.971	2.292	6.878	8.662×10^{-7}	A/G	0.157	0.046	SLC30A6
6	rs1773013	2560712	A	4942	3.277	2.041	5.261	9.011×10^{-7}	A/G	0.245	0.092	
2	rs2303553	182783653	C	4942	6.723	3.141	14.39	9.218×10^{-7}	C/T	0.078	0.012	SSFA2
2	rs77278954	182793839	A	4942	6.723	3.141	14.39	9.218×10^{-7}	A/G	0.078	0.012	SSFA2
2	rs78774163	182780126	A	4942	6.723	3.141	14.39	9.218×10^{-7}	A/G	0.078	0.012	SSFA2
8	rs74463341	9228334	C	4942	7.027	3.219	15.34	9.852×10^{-7}	C/G	0.078	0.014	
2	rs145475960	103130361	A	4942	5.499	2.778	10.89	9.995×10^{-7}	A/T	0.098	0.02	SLC9A4
2	2:102820009	102820009	G	4942	5.092	2.652	9.779	1.014×10^{-6}	G/C	0.108	0.024	IL1RL2
2	rs13419200	182758257	C	4942	6.666	3.115	14.27	1.026×10^{-6}	C/A	0.078	0.012	SSFA2
8	rs74382792	62356700	G	4942	6.941	3.181	15.15	1.134×10^{-6}	G/A	0.078	0.014	CLVS1
17	rs57769213	77879893	G	4942	7.199	3.251	15.94	1.138×10^{-6}	G/C	0.069	0.011	
20	rs140824800	12541106	A	4942	7.289	3.265	16.28	1.254×10^{-6}	A/G	0.069	0.011	
12	rs146647050	38191129	T	4942	5.931	2.871	12.25	1.514×10^{-6}	T/G	0.088	0.019	
7	rs142711375	46602409	G	4942	6.503	3.028	13.97	1.581×10^{-6}	G/A	0.078	0.014	
5	rs79287094	142892785	G	4942	8.409	3.522	20.08	1.624×10^{-6}	G/A	0.069	0.01	
9	rs150057407	3276207	G	4942	4.92	2.563	9.445	1.672×10^{-6}	G/T	0.108	0.024	RFX3
12	rs143302148	39100013	T	4942	7.507	3.286	17.15	1.739×10^{-6}	T/C	0.069	0.011	CPNE8
20	rs76232775	60768910	A	4942	4.44	2.408	8.186	1.789×10^{-6}	A/G	0.118	0.03	MTG2
23	rs149305693	27808447	C	4942	8.11	3.435	19.15	1.799×10^{-6}	C/T	0.069	0.012	
9	rs148123055	100176616	G	4942	6.626	3.044	14.42	1.886×10^{-6}	G/A	0.078	0.014	TDRD7
23	rs1433806	27812073	A	4942	8.08	3.421	19.08	1.887×10^{-6}	A/G	0.069	0.011	
12	rs150862851	38793434	G	4942	7.435	3.255	16.98	1.928×10^{-6}	G/A	0.069	0.012	
23	rs36115712	27825140	A	4942	8.066	3.415	19.05	1.931×10^{-6}	A/G	0.069	0.012	
23	rs146644158	27819452	T	4942	8.047	3.408	19	1.969×10^{-6}	T/C	0.069	0.012	
23	rs4829082	27805106	T	4942	8.047	3.408	19	1.969×10^{-6}	T/C	0.069	0.012	
23	rs6630571	27814160	A	4942	8.047	3.408	19	1.969×10^{-6}	A/G	0.069	0.012	
20	rs149264569	49715107	G	4942	6.037	2.878	12.66	1.971×10^{-6}	G/C	0.088	0.019	
6	rs9386997	111414038	A	4942	7.389	3.237	16.87	2.038×10^{-6}	A/T	0.069	0.011	SLC16A10
15	rs62026663	45485831	C	4942	3.221	1.987	5.221	2.060×10^{-6}	C/T	0.235	0.096	SHF

Table 3 (continued)

CHR	SNP	BP	Minor allele	N	OR	L95	U95	*p*	GTPS	MAF cases	MAF controls	Gene
9	rs187960516	36238454	A	4942	7.132	3.164	16.07	2.155×10^{-6}	A/G	0.069	0.01	CLTA-GNE
23	rs140339686	27830115	T	4942	7.963	3.372	18.81	2.226×10^{-6}	T/C	0.069	0.012	
23	rs4829084	27827112	A	4942	7.963	3.372	18.81	2.226×10^{-6}	A/G	0.069	0.012	
6	rs73010912	155067310	A	4942	6.174	2.903	13.13	2.265×10^{-6}	A/G	0.088	0.015	SCAF8
6	rs6921109	111448767	T	4942	7.309	3.203	16.68	2.297×10^{-6}	T/A	0.069	0.011	SLC16A10
6	rs7760668	111446502	C	4942	7.309	3.203	16.68	2.297×10^{-6}	C/A	0.069	0.011	SLC16A10
23	rs139460593	27817042	C	4942	7.925	3.357	18.71	2.319×10^{-6}	C/T	0.069	0.012	
6	rs72993420	155087077	G	4942	6.129	2.882	13.03	2.475×10^{-6}	G/A	0.088	0.015	SCAF8
15	rs62026667	45491136	G	4942	3.174	1.963	5.133	2.475×10^{-6}	G/C	0.235	0.097	SHF
15	rs142484525	95512720	T	4942	6.364	2.944	13.76	2.535×10^{-6}	T/A	0.069	0.011	

Top GWAS results based on 7,585,874 SNPs after imputation in 51 cases versus all 4891 population controls. All results were adjusted for genetic principal components 1–4. The threshold for statistical significance was $p < 5 \times 10^{-8}$

GWAS genome-wide association study, *CHR* chromosome, *SNP* single nucleotide polymorphism, *BP* base pair, *N* number, *GTPS* Guanosine-5′-triphosphates, *MAF* minor allele frequency, *OR [95% CI]* odds ratio with 95% confidence interval, *p p* value

Candidate Gene Analyses—Cases Versus Matched Controls

When cases of bisphosphonate-associated AFF were compared with matched controls, no statistically significant associations were revealed (Fig. 2b; Table 6; Supplemental Table 2).

Discussion

We were hoping to find a strong common genetic susceptibility trait for AFF to predict patients at high risk of this ADR. Our results indicate that there is no common genetic variant that can be used for this purpose. The only significant finding on a genome-wide level was with four SNPs when cases were compared with population controls, but these were uncommon SNPs, all of which were single hits, meaning that these associations are likely false positives [24, 25], although two may theoretically be related to the treatment indication (NR3C1 and NTN1). None of these specific SNPs have, however, previously been implicated in AFF or osteoporosis [11, 26–28]. After reducing the risk of confounding by indication with the use of a comparison to bisphosphonate-treated controls, no statistically significant association remained.

At this time we are therefore left to models based on pharmacological and clinical considerations to minimize the risk of AFF. The prevailing pathophysiological theory of AFF is that bisphosphonates lead to over-suppression of bone remodeling [29]. Because bisphosphonates preferentially suppress the targeted repair mechanism, increased numbers of micro-cracks and reduced heterogeneity of the bone can be seen in bone tissue from animals and humans [7, 30–32]. The combination of these can lead to accumulation of micro-cracks during normal loading and propagation to larger cracks, eventually resulting in complete AFF. Studies have shown that the risk of developing an AFF is on average 50-fold greater for a bisphosphonate user compared to a nonuser, and more than 100-fold greater after 4–5 years of treatment [3, 5, 33]. In contrast, discontinuation of the drug will lead to a steep decline in the risk for developing an AFF [3]. In addition, different bisphosphonates might vary in terms of risk [3, 5, 34]. Hence, treatment duration and choice of bisphosphonate could be subject to manipulation in order to gain maximum treatment benefit while reducing the risk of AFF.

Many attempts have been made to identify risk factors that may predispose bisphosphonate users to AFF. A potential genetic influence has been suggested as a possible explanation to why only a minority of bisphosphonate users develop AFF. For instance, studies have revealed that polymorphisms in the gene encoding farnesyl diphosphate synthase (FDPS) may affect bone mineral density and bone turnover following bisphosphonate treatment in some patients, while not in others [35–38]. A possible genetic cause is also supported by studies that have demonstrated a difference in risk of AFF based on ethnicity, with Asians being at higher risk. A recent study by Lo et al. revealed a hazard ratio of 6.6 for females of Asian ethnicity compared with Caucasian women [9]. In addition, theories of a possible genetic trait have been long existing for other bisphosphonate ADRs that manifest in the skeleton [39].

There are several limitations to this study. First, matching of controls was done using bisphosphonate exposure as a proxy for osteoporosis as the Swedish Patient Register mainly includes information on diagnoses from hospital

Table 4 Top genome-wide associations with bisphosphonate-associated atypical femoral fractures—cases vs matched controls

CHR	SNP	BP	Minor allele	N	OR	L95	U95	*p*	GTPS	MAF case	MAF control	Gene
16	rs7188484	88918607	T	375	3.576	2.153	5.94	8.605×10^{-7}	T/G	0.431	0.196	GALNS
3	rs6768500	2693258	C	375	7.634	3.379	17.25	1.021×10^{-6}	C/G	0.157	0.023	CNTN4
3	rs76646538	2694727	C	375	7.634	3.379	17.25	1.021×10^{-6}	C/T	0.157	0.023	CNTN4
1	rs1913592	18550837	C	375	3.346	2.06	5.435	1.055×10^{-6}	C/T	0.529	0.279	IGSF21
12	rs4765913	2419896	A	375	3.114	1.942	4.995	2.454×10^{-6}	A/T	0.412	0.188	CACNA1C
16	rs12444242	88911043	T	375	3.269	1.987	5.38	3.125×10^{-6}	T/C	0.402	0.182	GALNS
16	rs12447646	88910824	A	375	3.269	1.987	5.38	3.125×10^{-6}	A/G	0.402	0.182	GALNS
16	rs12449164	88909788	T	375	3.269	1.987	5.38	3.125×10^{-6}	T/C	0.402	0.182	GALNS
16	rs8054592	88912039	T	375	3.269	1.987	5.38	3.125×10^{-6}	T/C	0.402	0.182	GALNS
16	rs12932521	88914235	T	375	3.242	1.97	5.335	3.679×10^{-6}	T/C	0.402	0.184	GALNS
16	rs34858110	88914598	C	375	3.242	1.97	5.335	3.679×10^{-6}	C/A	0.402	0.184	GALNS
16	rs71395332	88909028	T	375	3.243	1.97	5.336	3.683×10^{-6}	T/C	0.402	0.184	GALNS
16	rs12598981	88916036	T	375	3.217	1.955	5.293	4.278×10^{-6}	T/G	0.402	0.185	GALNS
16	rs11076726	88912899	T	375	3.219	1.953	5.306	4.503×10^{-6}	T/G	0.422	0.201	GALNS
8	rs17063092	3104832	C	375	2.958	1.86	4.703	4.614×10^{-6}	C/T	0.461	0.238	CSMD1
7	rs12538221	24123003	T	375	5.237	2.575	10.65	4.867×10^{-6}	T/C	0.167	0.045	
7	rs71526045	24118952	A	375	5.237	2.575	10.65	4.867×10^{-6}	A/G	0.167	0.045	
17	rs61753147	8809025	A	375	5.265	2.58	10.74	4.995×10^{-6}	A/G	0.167	0.035	PIK3R5
16	rs34495980	88906555	A	375	3.177	1.93	5.232	5.544×10^{-6}	A/C	0.402	0.188	GALNS
15	rs4776851	67180920	A	375	6.06	2.776	13.23	6.075×10^{-6}	A/G	0.137	0.031	
16	16:88906780	88906780	G	375	3.152	1.914	5.191	6.427×10^{-6}	G/A	0.402	0.19	GALNS
16	rs13337256	88907043	G	375	3.152	1.914	5.191	6.427×10^{-6}	G/A	0.402	0.19	GALNS
16	rs3784881	88905888	T	375	3.152	1.914	5.191	6.427×10^{-6}	T/C	0.402	0.19	GALNS
18	rs116941264	75460371	A	375	10.78	3.833	30.33	6.609×10^{-6}	A/G	0.098	0.011	
7	rs2727797	36628761	T	375	3.142	1.909	5.169	6.613×10^{-6}	T/C	0.676	0.44	AOAH
10	rs7082862	134341963	G	375	3.851	2.139	6.93	6.916×10^{-6}	G/C	0.226	0.071	
2	rs11465606	102988300	A	375	6.86	2.958	15.91	7.252×10^{-6}	A/C	0.128	0.022	IL18R1
8	rs17319624	3105800	A	375	3.161	1.906	5.242	8.180×10^{-6}	A/G	0.363	0.176	CSMD1
10	rs36009580	73627786	G	375	2.965	1.839	4.78	8.200×10^{-6}	G/C	0.412	0.194	
3	rs2717296	182456980	C	375	2.864	1.802	4.552	8.603×10^{-6}	C/T	0.686	0.426	
8	rs17319596	3104594	C	375	2.846	1.795	4.513	8.681×10^{-6}	C/T	0.461	0.245	CSMD1
8	rs17319617	3105038	A	375	3.103	1.878	5.126	9.811×10^{-6}	A/C	0.363	0.176	CSMD1
8	rs34162586	3105087	C	375	3.103	1.878	5.126	9.811×10^{-6}	C/G	0.363	0.176	CSMD1
8	rs35729878	3104896	G	375	3.103	1.878	5.126	9.811×10^{-6}	G/C	0.363	0.176	CSMD1
15	rs62026663	45485831	C	375	3.643	2.054	6.463	9.814×10^{-6}	C/T	0.235	0.083	SHF
8	8:3104001	3104001	T	375	3.578	2.032	6.3	1.007×10^{-5}	T/G	0.245	0.096	CSMD1
8	rs117459261	3103995	T	375	3.578	2.032	6.3	1.007×10^{-5}	T/A	0.245	0.096	CSMD1
8	rs73185574	3106144	C	375	3.099	1.874	5.124	1.041×10^{-5}	C/T	0.363	0.179	CSMD1
8	rs142418205	3097543	G	375	3.218	1.912	5.418	1.093×10^{-5}	G/A	0.343	0.167	CSMD1
16	rs8062286	88917502	A	375	3.102	1.873	5.138	1.101×10^{-5}	A/G	0.402	0.198	GALNS
7	rs3801298	36569019	T	375	3.098	1.87	5.131	1.123×10^{-5}	T/C	0.716	0.486	AOAH
18	rs3016811	589690	T	375	2.609	1.7	4.002	1.126×10^{-5}	T/C	0.628	0.381	
18	rs518302	589635	G	375	2.609	1.7	4.002	1.126×10^{-5}	G/A	0.628	0.381	
2	rs6723676	22414978	A	375	2.821	1.774	4.484	1.159×10^{-5}	A/C	0.559	0.327	
20	rs149264569	49715107	G	375	10.89	3.744	31.68	1.170×10^{-5}	G/C	0.088	0.011	
4	rs116838635	112534842	A	375	5.704	2.617	12.43	1.187×10^{-5}	A/G	0.137	0.034	
17	rs111859148	32210110	C	375	3.174	1.893	5.323	1.191×10^{-5}	C/T	0.304	0.13	ASIC2
17	rs2348157	32210243	G	375	3.174	1.893	5.323	1.191×10^{-5}	G/C	0.304	0.13	ASIC2
17	rs56174865	32214269	A	375	3.174	1.893	5.323	1.191×10^{-5}	A/G	0.304	0.13	ASIC2
17	rs66923090	32215593	A	375	3.174	1.893	5.323	1.191×10^{-5}	A/G	0.304	0.13	ASIC2

Table 4 (continued)

CHR	SNP	BP	Minor allele	N	OR	L95	U95	*p*	GTPS	MAF case	MAF control	Gene
17	rs67026511	32215830	G	375	3.174	1.893	5.323	1.191×10^{-5}	G/A	0.304	0.13	ASIC2
17	rs67236820	32215903	A	375	3.174	1.893	5.323	1.191×10^{-5}	A/G	0.304	0.13	ASIC2
17	rs67809660	32215544	C	375	3.174	1.893	5.323	1.191×10^{-5}	C/T	0.304	0.13	ASIC2
17	rs68033423	32215432	C	375	3.174	1.893	5.323	1.191×10^{-5}	C/T	0.304	0.13	ASIC2
17	rs68085213	32215389	C	375	3.174	1.893	5.323	1.191×10^{-5}	C/T	0.304	0.13	ASIC2
17	rs72818938	32215882	C	375	3.174	1.893	5.323	1.191×10^{-5}	C/T	0.304	0.13	ASIC2
17	rs8069564	32215953	T	375	3.174	1.893	5.323	1.191×10^{-5}	T/C	0.304	0.13	ASIC2
17	rs8070346	32212347	C	375	3.174	1.893	5.323	1.191×10^{-5}	C/G	0.304	0.13	ASIC2
17	rs8074055	32215922	C	375	3.174	1.893	5.323	1.191×10^{-5}	C/T	0.304	0.13	ASIC2
17	rs8076707	32212839	C	375	3.174	1.893	5.323	1.191×10^{-5}	C/T	0.304	0.13	ASIC2

Top GWAS results based on 7,585,874 SNPs after imputation in 51 cases versus 324 matched controls. All results were adjusted for genetic principal components 1–4. The threshold for statistical significance was $p < 5 \times 10^{-8}$

GWAS genome-wide association study, *CHR* chromosome, *SNP* single nucleotide polymorphism, *BP* base pair, *N* number, *GTPS* Guanosine-5′-triphosphates, *MAF* minor allele frequency, *OR [95% CI]* odds ratio with 95% confidence interval, *p p* value

care. We were thus unable to identify controls who were prescribed a bisphosphonate for osteoporosis prevention. Secondly, although this is the largest genetic study of bisphosphonate-associated AFF to date, the number of included cases is still low. This means that the power to detect weakly associated common variants and strongly associated rare variants is low. It is also possible that several variants, inherited independently of one another, are required to infer a risk of AFF, in which case they will go undetected. To elucidate this would require a larger study and whole genome or exome sequencing, which was beyond the scope of this study. Lastly, there are suggestions that the association between bisphosphonate use and AFF is mainly driven by a genetic predisposition [11]. However, since 4–5 years of bisphosphonate use in Swedish women is associated with a 125-fold increase in risk of AFF [3], the potential underlying causal genetic risk allele(-s) should have a firm relation with both AFF and bisphosphonate use to entirely extenuate the exponential increase in risk with duration of bisphosphonate use. Noteworthily, a more moderately strong effect modification between bisphosphonates and genetic predisposition might still exist, but the current study is too small to disentangle such genetic modifying effects.

That several genetic loci, perhaps varying between individuals, might explain at least some cases of bisphosphonate-associated AFF has been proposed by some studies, although methodological issues and other limitations makes it difficult to conclude whether the findings are of relevance for a larger population of individuals with bisphosphonate-associated AFF. In the study by Pérez-Núñez et al. that compared 13 women with AFF and 268 female controls, 21 loci were more frequent in the fracture group [40]. Most patients accumulated two or more allelic variants, and the number of variants was different between patients with fractures and the controls, suggesting that several genes may be involved. The study was, however, limited by the fact that the controls were a mix of normal and osteoporotic women, and that only 12 of the 13 cases had been exposed to bisphosphonates. In another study, Roca-Ayats et al. performed whole-exome sequencing in three sisters who had all developed AFF following bisphosphonate treatment, and compared with three unrelated patients with bisphosphonate-associated AFF [41]. They detected 37 rare nonsynonymous mutations in 34 genes, but the results are questionable due to lack of validation and a small sample size. In a further study, Funck-Brentano et al. performed sequencing of four genes amongst two patients with bisphosphonate-associated AFF and found genetic variants in one, a rare heterozygous mutation in COL1A2 (c.213G > A; p.Arg708GIn) [42]. Limitations of this study include the small sample size. While these findings suggest a polygenic model in which an accumulation of susceptibility variants may lead to a predisposition to bisphosphonate-associated AFF, larger studies are required to provide solid evidence.

Conclusion

With this genome-wide association and candidate gene study, we were unable to find evidence of common genetic traits predisposition for bisphosphonate-associated AFF. This does not rule out the possibility of weakly associated genetic traits or the presence of rare genetic variants that confer a risk. Further studies of larger sample size as well as whole-exome or whole-genome sequencing studies are warranted.

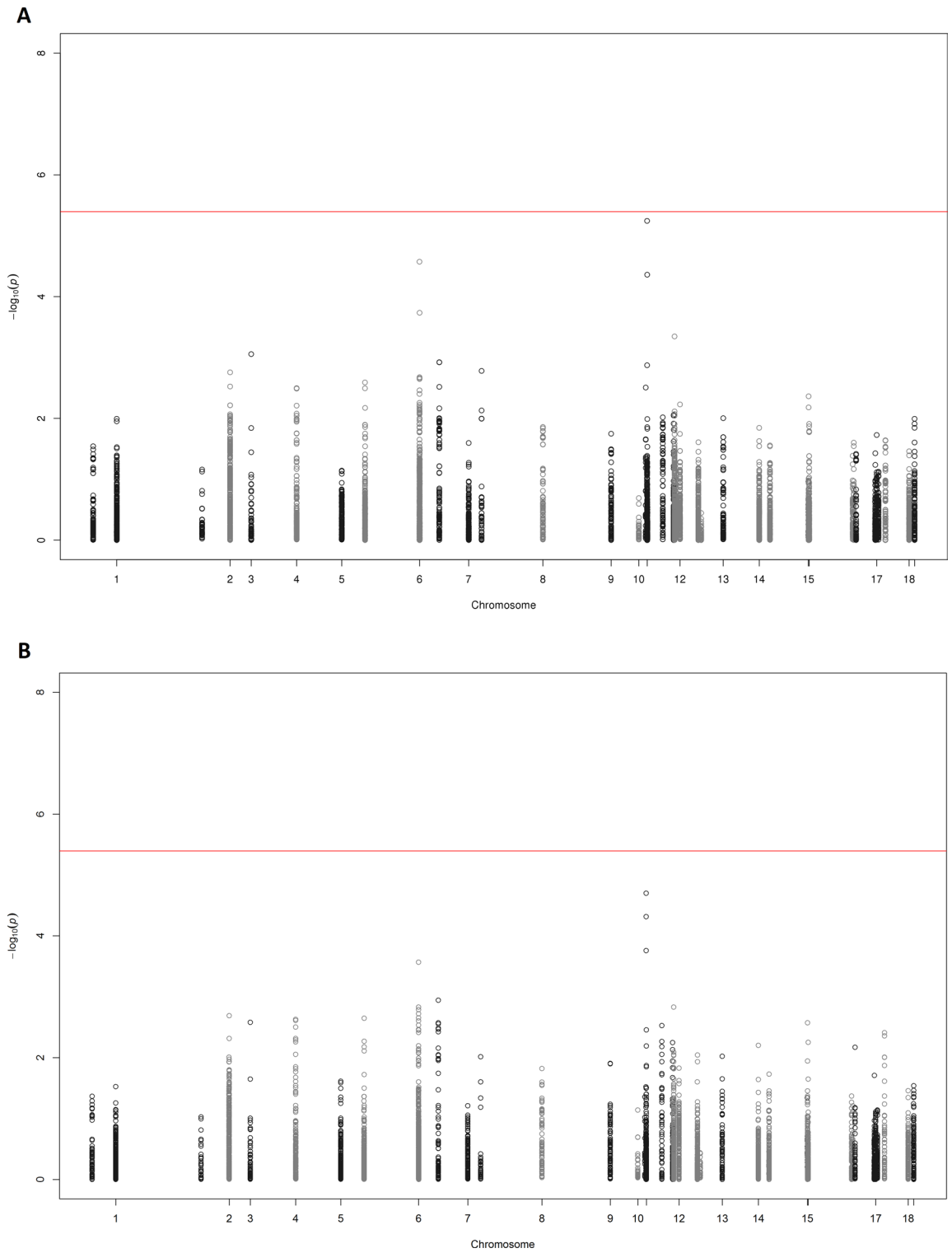

Fig. 2 **a** Manhattan plot of the candidate gene analyses—cases vs all 4891 controls. **b** Manhattan plot of the candidate gene analyses—cases vs matched controls. Analyses of 51 cases of bisphosphonate-associated atypical femoral fractures versus **a** all 4891 controls, and **b** 324 matched controls. Adjustment was made for genetic principal components 1–4. The red line shows the threshold for statistical significance of 5.74×10^{-6}. There were no statistically significant associations in either analysis

Table 5 Top candidate gene associations with bisphosphonate-associated atypical femoral fractures

CHR	SNP	BP	Minor allele	N	OR	L95	U95	*p*	GTPS	MAF cases	MAF controls	Gene
2	rs181660819	111578634	G	4942	5.42	2.284	12.87	1.271×10^{-4}	G/A	0.059	0.013	ACOXL
5	rs116741837	169450719	T	4942	5.474	2.28	13.14	1.425×10^{-4}	T/C	0.059	0.013	DOCK2
16	rs17821406	57919041	T	4942	2.713	1.612	4.566	1.721×10^{-4}	T/C	0.167	0.068	CNGB1
2	rs138252364	111483994	C	4942	3.573	1.756	7.272	4.435×10^{-4}	C/G	0.088	0.027	
16	rs12446558	57915370	A	4942	2.533	1.484	4.325	6.607×10^{-4}	A/T	0.157	0.068	
10	rs116907192	106148123	T	4942	4.48	1.811	11.08	1.172×10^{-3}	T/C	0.049	0.012	CCDC147
2	rs140272071	111510669	A	4942	3.247	1.592	6.62	1.197×10^{-3}	A/G	0.088	0.03	ACOXL
2	rs3789117	111712123	C	4942	2.15	1.342	3.443	1.447×10^{-3}	C/T	0.235	0.128	ACOXL
16	rs116919349	57911019	G	4942	2.323	1.361	3.966	1.998×10^{-3}	G/A	0.157	0.074	
16	rs17240952	57910443	C	4942	2.322	1.36	3.964	2.013×10^{-3}	C/T	0.157	0.074	
5	rs10063658	169131347	T	4942	3.151	1.52	6.531	2.035×10^{-3}	T/C	0.088	0.026	DOCK2
5	rs111717777	169128756	G	4942	3.103	1.497	6.435	2.336×10^{-3}	G/A	0.088	0.027	DOCK2
16	rs79806773	57917473	C	4942	2.283	1.34	3.891	2.396×10^{-3}	C/G	0.157	0.075	
9	rs76038546	136345878	C	4942	2.614	1.401	4.877	2.543×10^{-3}	C/A	0.118	0.052	
9	9:136352590	136352590	T	4942	2.567	1.38	4.775	2.903×10^{-3}	T/C	0.118	0.052	
1	rs114420253	248103804	A	4942	2.896	1.432	5.856	3.071×10^{-3}	A/G	0.088	0.034	OR2L13
5	rs262864	169200927	A	4942	1.999	1.263	3.163	3.094×10^{-3}	A/G	0.255	0.142	DOCK2-
9	9:136338187	136338187	C	4942	0.218	0.0786	0.602	3.312×10^{-3}	C/A	0.039	0.148	SLC2A6
10	rs117846723	106186205	A	4942	3.646	1.53	8.691	3.514×10^{-3}	A/C	0.059	0.018	CCDC147
2	rs55739979	216234981	C	4942	3.244	1.469	7.162	3.593×10^{-3}	C/G	0.069	0.022	FN1
5	rs116213385	169457689	T	4942	3.561	1.515	8.372	3.595×10^{-3}	T/A	0.059	0.018	DOCK2
2	rs3827546	111718499	C	4942	1.988	1.242	3.183	4.219×10^{-3}	C/G	0.235	0.136	ACOXL
2	rs3789119	111707405	T	4942	1.81	1.205	2.719	4.283×10^{-3}	T/C	0.372	0.25	ACOXL
5	rs114254961	169213503	A	4942	3.425	1.455	8.059	4.813×10^{-3}	A/G	0.059	0.019	DOCK2
10	rs117402638	106199934	G	4942	3.428	1.449	8.111	5.04×10^{-3}	G/T	0.059	0.02	CCDC147
7	7:94036547	94036547	T	4942	1.728	1.172	2.548	5.772×10^{-3}	T/C	0.461	0.326	COL1A2
2	2:111621582	111621582	G	4942	2.274	1.265	4.09	6.061×10^{-3}	G/T	0.137	0.067	ACOXL
5	rs76019338	169229582	A	4942	1.878	1.197	2.946	6.108×10^{-3}	A/G	0.265	0.155	DOCK2
15	rs116916068	74920220	A	4942	2.311	1.265	4.222	6.427×10^{-3}	A/G	0.128	0.061	CLK3
5	rs12520941	169218189	T	4942	1.867	1.19	2.93	6.606×10^{-3}	T/G	0.265	0.156	DOCK2
2	rs74791643	111823562	G	4942	4.237	1.493	12.03	6.68×10^{-3}	G/A	0.039	0.011	ACOXL
5	rs76621262	169356148	C	4942	4.081	1.477	11.28	6.686×10^{-3}	C/G	0.039	0.011	DOCK2-FAM196B
2	rs2670632	111586327	T	4942	1.708	1.157	2.521	7.042×10^{-3}	T/G	0.471	0.334	ACOXL
1	rs72763242	248187347	A	4942	3.607	1.408	9.236	7.502×10^{-3}	A/G	0.049	0.015	OR2L13
2	rs3789100	111731713	C	4942	1.887	1.18	3.017	8.03×10^{-3}	C/T	0.226	0.135	ACOXL
2	rs7564385	111734779	T	4942	1.887	1.18	3.017	8.03×10^{-3}	T/C	0.226	0.135	ACOXL

Table 5 (continued)

CHR	SNP	BP	Minor allele	N	OR	L95	U95	*p*	GTPS	MAF cases	MAF controls	Gene
1	rs4654971	21897903	C	4942	2.261	1.235	4.141	8.226×10^{-3}	C/T	0.118	0.055	ALPL
1	rs3738098	21894785	T	4942	2.256	1.232	4.133	8.432×10^{-3}	T/G	0.118	0.055	ALPL
2	rs11687442	216246210	G	4942	1.726	1.148	2.595	8.653×10^{-3}	G/T	0.392	0.272	FN1
1	1:21903180	21903180	T	4942	2.242	1.223	4.108	8.987×10^{-3}	T/C	0.118	0.055	ALPL
2	rs3789101	111729489	C	4942	1.868	1.168	2.989	9.099×10^{-3}	C/G	0.226	0.136	ACOXL
2	rs12694363	216254032	A	4942	1.694	1.139	2.519	9.227×10^{-3}	A/G	0.441	0.316	FN1
16	rs117529794	58005931	T	4942	3.933	1.387	11.15	0.01001	T/C	0.039	0.011	
5	rs10462993	169497539	A	4942	1.757	1.143	2.701	0.01015	A/G	0.284	0.183	DOCK2
1	rs2242421	21904574	G	4942	2.15	1.199	3.856	0.01022	G/A	0.137	0.067	ALPL
1	rs7533989	210801954	G	4942	1.693	1.132	2.53	0.01029	G/C	0.412	0.296	HHAT
7	rs3750109	94042814	C	4942	1.907	1.163	3.126	0.01052	C/T	0.206	0.115	COL1A2
5	rs112139518	169198357	A	4942	2.562	1.245	5.272	0.01059	A/G	0.088	0.032	DOCK2
17	rs76141655	80570428	A	4942	2.777	1.264	6.101	0.01099	A/G	0.069	0.028	FOXK2
16	rs79070935	70578817	A	4942	3.157	1.296	7.689	0.01137	A/C	0.049	0.015	SF3B3
5	rs10462992	169497534	T	4942	1.738	1.13	2.672	0.01189	T/C	0.284	0.185	DOCK2
16	rs411657	57941094	T	4942	0.586	0.387	0.889	0.01198	T/C	0.333	0.461	CNGB1
10	rs11202848	90532166	A	4942	2.174	1.185	3.989	0.01211	A/C	0.118	0.057	LIPN
10	rs11202852	90544073	A	4942	2.174	1.185	3.989	0.01211	A/G	0.118	0.057	
10	rs12572022	90545882	A	4942	2.174	1.185	3.989	0.01211	A/C	0.118	0.057	RCBTB2P1
10	rs17112679	90527569	C	4942	2.174	1.185	3.989	0.01211	C/T	0.118	0.057	LIPN
10	rs11202853	90545416	A	4942	2.17	1.183	3.982	0.01234	A/G	0.118	0.057	RCBTB2P1
5	rs264838	169134768	T	4942	2.496	1.216	5.124	0.01264	T/C	0.088	0.033	DOCK2
16	rs17240980	57933771	C	4942	2.094	1.171	3.745	0.01268	C/T	0.137	0.071	CNGB1
5	rs73318247	169155152	T	4942	2.492	1.214	5.116	0.01284	T/G	0.088	0.033	DOCK2

Top results after imputation in 51 cases versus all 4891 controls. All results were adjusted for genetic principal components 1–4. The threshold for statistical significance was $p < 5.74 \times 10^{-6}$

GWAS genome-wide association study, *CHR* chromosome, *SNP* single nucleotide polymorphism, *BP* base pair, *N* number, *GTPS* Guanosine-5′-triphosphates, *MAF* minor allele frequency, *OR [95% CI]* odds ratio with 95% confidence interval, *p p* value

Table 6 Top candidate gene associations with bisphosphonate-associated atypical femoral fractures

CHR	SNP	BP	Minor allele	N	OR	L95	U95	p	GTPS	MAF cases	MAF controls	Gene
9	9:136352590	136352590	T	375	5.239	2.305	11.91	7.72×10^{-5}	T/C	0.118	0.029	
9	rs76038546	136345878	C	375	4.854	2.165	10.88	1.254×10^{-4}	C/A	0.118	0.031	
2	rs138252364	111483994	C	375	5.002	1.983	12.61	6.47×10^{-4}	C/G	0.088	0.02	
2	rs140272071	111510669	A	375	4.58	1.838	11.41	1.09×10^{-3}	A/G	0.088	0.022	ACOXL
5	rs262864	169200927	A	375	2.337	1.395	3.915	1.269×10^{-3}	A/G	0.255	0.117	DOCK2-
2	2:111621582	111621582	G	375	3.334	1.599	6.951	1.319×10^{-3}	G/T	0.137	0.049	ACOXL
10	rs116907192	106148123	T	375	8.746	2.298	33.28	1.47×10^{-3}	T/C	0.049	0.008	CCDC147
2	rs181660819	111578634	G	375	6.45	2.037	20.42	1.524×10^{-3}	G/A	0.059	0.011	ACOXL
5	rs114254961	169213503	A	375	6.187	2	19.14	1.56×10^{-3}	A/G	0.059	0.015	DOCK2
1	1:21877265	21877265	C	375	3.204	1.525	6.731	2.115×10^{-3}	C/G	0.118	0.045	ALPL
1	rs113561139	21909239	C	375	3.158	1.507	6.619	2.316×10^{-3}	C/G	0.118	0.046	
11	rs78214094	4909900	C	375	13.59	2.376	77.71	3.364×10^{-3}	C/T	0.039	0.003	MMP26
2	rs3789106	111720884	G	375	1.871	1.229	2.847	3.453×10^{-3}	G/T	0.51	0.363	ACOXL
2	rs13003263	111710045	T	375	0.5213	0.3336	0.8147	4.244×10^{-3}	T/C	0.382	0.532	ACOXL
2	rs3789115	111712251	A	375	0.5213	0.3336	0.8147	4.244×10^{-3}	A/G	0.382	0.532	ACOXL
2	rs4577288	111713046	T	375	0.5213	0.3336	0.8147	4.244×10^{-3}	T/G	0.382	0.532	ACOXL
2	rs6750439	111711536	T	375	0.5213	0.3336	0.8147	4.244×10^{-3}	T/C	0.382	0.532	ACOXL
1	rs114420253	248103804	A	375	3.541	1.487	8.432	4.281×10^{-3}	A/G	0.088	0.031	OR2L13
2	rs1877655	111712703	C	375	0.5211	0.3331	0.8152	4.308×10^{-3}	C/T	0.372	0.523	ACOXL
2	rs2341914	111713724	T	375	0.5211	0.3331	0.8152	4.308×10^{-3}	T/C	0.372	0.523	ACOXL
2	rs2341915	111713661	T	375	0.5211	0.3331	0.8152	4.308×10^{-3}	T/C	0.372	0.523	ACOXL
2	rs2880190	111713595	T	375	0.5211	0.3331	0.8152	4.308×10^{-3}	T/A	0.372	0.523	ACOXL
2	rs4619626	111713057	T	375	0.5211	0.3331	0.8152	4.308×10^{-3}	T/C	0.372	0.523	ACOXL
9	9:136338187	136338187	C	375	0.2256	0.08075	0.6302	4.498×10^{-3}	C/A	0.039	0.156	SLC2A6
1	rs116121521	21876957	C	375	2.892	1.388	6.027	4.585×10^{-3}	C/T	0.118	0.049	ALPL
2	rs11687442	216246210	G	375	1.926	1.223	3.034	4.69×10^{-3}	G/T	0.392	0.258	FN1
5	rs111913365	169447265	G	375	6.491	1.769	23.82	4.807×10^{-3}	G/A	0.049	0.009	DOCK2
5	rs76469325	169447222	T	375	6.491	1.769	23.82	4.807×10^{-3}	T/G	0.049	0.009	DOCK2
5	rs116741837	169450719	T	375	4.669	1.595	13.66	4.918×10^{-3}	T/C	0.059	0.019	DOCK2
2	rs112273617	233841768	C	375	9.255	1.96	43.71	4.966×10^{-3}	C/T	0.039	0.005	NGEF
2	rs149536245	111709828	G	375	9.066	1.939	42.4	5.095×10^{-3}	G/A	0.039	0.005	ACOXL
1	rs141276685	21888425	A	375	2.836	1.363	5.904	5.313×10^{-3}	A/G	0.118	0.051	ALPL
15	rs116916068	74920220	A	375	2.647	1.32	5.31	6.104×10^{-3}	A/G	0.128	0.052	CLK3
2	rs3789119	111707405	T	375	1.862	1.189	2.918	6.643×10^{-3}	T/C	0.372	0.253	ACOXL
10	rs10887854	90540941	G	375	2.884	1.342	6.2	6.677×10^{-3}	G/A	0.118	0.051	
10	rs10887855	90541206	T	375	2.884	1.342	6.2	6.677×10^{-3}	T/C	0.118	0.051	

Table 6 (continued)

CHR	SNP	BP	Minor allele	N	OR	L95	U95	*p*	GTPS	MAF cases	MAF controls	Gene
10	rs11202848	90532166	A	375	2.884	1.342	6.2	6.677×10^{-3}	A/C	0.118	0.051	LIPN
10	rs11202850	90535654	G	375	2.884	1.342	6.2	6.677×10^{-3}	G/T	0.118	0.051	LIPN
10	rs11202851	90537942	T	375	2.884	1.342	6.2	6.677×10^{-3}	T/C	0.118	0.051	LIPN
10	rs11202852	90544073	A	375	2.884	1.342	6.2	6.677×10^{-3}	A/G	0.118	0.051	
10	rs11202855	90547504	A	375	2.884	1.342	6.2	6.677×10^{-3}	A/G	0.118	0.051	
10	rs12572022	90545882	A	375	2.884	1.342	6.2	6.677×10^{-3}	A/C	0.118	0.051	RCBTB2P1
10	rs17112679	90527569	C	375	2.884	1.342	6.2	6.677×10^{-3}	C/T	0.118	0.051	LIPN
10	rs17112704	90529566	T	375	2.884	1.342	6.2	6.677×10^{-3}	T/A	0.118	0.051	LIPN
2	rs71431135	111809400	G	375	0.1615	0.04269	0.6107	7.222×10^{-3}	G/A	0.029	0.119	ACOXL
7	7:94036547	94036547	T	375	1.779	1.166	2.716	7.547×10^{-3}	T/C	0.461	0.31	COL1A2
2	rs13024581	111823835	C	375	0.1635	0.0432	0.6186	7.648×10^{-3}	C/T	0.029	0.119	ACOXL
2	rs2118908	111824592	G	375	0.1635	0.0432	0.6186	7.648×10^{-3}	G/A	0.029	0.119	ACOXL
2	rs71431138	111818383	C	375	0.1635	0.0432	0.6186	7.648×10^{-3}	C/T	0.029	0.119	ACOXL
2	rs13034863	111810020	G	375	0.1646	0.04355	0.6224	7.842×10^{-3}	G/C	0.029	0.117	ACOXL
2	rs34121532	111810633	G	375	0.1646	0.04355	0.6224	7.842×10^{-3}	G/A	0.029	0.117	ACOXL
2	rs35875858	111811106	G	375	0.1646	0.04355	0.6224	7.842×10^{-3}	G/C	0.029	0.117	ACOXL
2	rs36091399	111810844	T	375	0.1646	0.04355	0.6224	7.842×10^{-3}	T/G	0.029	0.117	ACOXL
2	rs71431134	111808175	G	375	0.1646	0.04355	0.6224	7.842×10^{-3}	G/C	0.029	0.117	ACOXL
2	rs71431136	111809837	C	375	0.1646	0.04355	0.6224	7.842×10^{-3}	C/T	0.029	0.117	ACOXL
2	rs78210391	216273212	A	375	6.618	1.64	26.71	7.943×10^{-3}	A/C	0.039	0.008	FN1
2	rs17483962	111826292	C	375	0.1672	0.04419	0.6328	8.441×10^{-3}	C/G	0.029	0.117	ACOXL
2	rs17549841	111826389	T	375	0.1672	0.04419	0.6328	8.441×10^{-3}	T/C	0.029	0.117	ACOXL
2	rs35812219	111826286	T	375	0.1672	0.04419	0.6328	8.441×10^{-3}	T/C	0.029	0.117	ACOXL
2	rs74848138	111825521	G	375	0.1672	0.04419	0.6328	8.441×10^{-3}	G/A	0.029	0.117	ACOXL

Top results after imputation in 51 cases versus 324 matched controls. All results were adjusted for genetic principal components 1–4. The threshold for statistical significance was $p < 5.74 \times 10^{-6}$

GWAS genome-wide association study, *CHR* chromosome, *SNP* single-nucleotide polymorphism, *BP* base pair, *N* number, *GTPS* Guanosine-5′-triphosphates, *MAF* minor allele frequency, *OR [95% CI]* odds ratio with 95% confidence interval, *p p* value

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Author Contributions Study design: PH and MW. Data collection: MK, MW, KM, JS and PH. Data analysis: NE. Data interpretation: MK, PH, MW, KM, JS, HM and NE. Drafting manuscript: MK and PH. Revising manuscript content: MK, PH, MW, KM, JS, HM and NE. Approving final version of manuscript: MK, PH, MW, KM, JS, HM and NE.

Compliance with Ethical Standards

Conflict of interest Mohammad Kharazmi, Karl Michaëlsson, Jörg Schilcher, Niclas Eriksson, Håkan Melhus, Mia Wadelius, and Pär Hallberg declare that they have no conflict of interest.

Ethical Approval The study was approved by the regional ethical review boards in Uppsala and Stockholm (2010/231 in Uppsala; 2007/644-31 and 2011/463-32 in Stockholm).

Informed Consent Written informed consent was obtained from all participants.

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